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Next-generation chemical biology: Mapping the Purinome druggable space reveals genotype-specific vulnerabilities via phenotypic screening

Background: Phenotypic screening enables discovery of biologically active compounds without prior knowledge of specific molecular targets. When combined with systematic chemical-space analysis, it represents a next-generation framework for mapping the druggable space across diverse genetic backgrounds. The human Purinome - a family of purine-interacting proteins composed of kinases and other nucleotide-binding enzymes - contains many attractive drug targets. Here, we applied our ATLAS (Active Learning with Automated Synthesis and Screening) platform which integrates high-throughput precision chemistry, direct to biology (D2B) screening, and machine learning, to survey the Purinome druggable space using diverse purine-directed libraries, aiming to identify genotype-specific dependencies within this critical protein network.

Methods: Using ATLAS, we generated proprietary, structurally diverse small molecule libraries of over 100,000 purine-directed samples, synthesized at nanoliter scale and directly screened in a high-throughput 1536-well cell viability assay. Screening was conducted in two colorectal cancer cell lines with distinct genotypes: HCT-116 and NCI-H747. Active D2B hits were validated by full dose-response curves, followed by pure resynthesis to confirm activity, selectivity, and potency. High-resolution chemical-space analysis was applied to visualize the diversity of active compounds, map activity across the surveyed chemical space, and define structure-activity relationships (SAR), enabling the identification of uniquely active scaffolds.

Results: ATLAS enabled exploration of a diverse chemical space which yielded thousands of cell-active compounds, many with strong cell line selectivity. Iterative application of ATLAS rapidly improved potency. As SAR was refined through the triage, hits clustered into distinct, non-overlapping chemical scaffolds, suggesting engagement with different biological targets. Importantly, activity maps differed between the two cell lines, revealing genotype-specific dependencies. One prominent chemotype contained structurally related molecules to known Aurora kinase inhibitors with clear cell line selectivity, while others represent novel, uncharacterized dependencies. These results demonstrate the platform's efficiency in simultaneously discovering genotype-selective, cell-active compounds and accelerating their refinement toward optimal biological activity.

Conclusion: Using ATLAS, we performed phenotypic screening combined with high-resolution mapping of Purinome-directed chemical space. This approach identified small molecule compounds linked to genotype-specific vulnerabilities, establishing ATLAS as a powerful strategy for dissecting the druggable proteome and uncovering genotype-tailored therapeutic targets.